Cattail phenology and hybrid incidence at the Olentangy River Wetland Research Park

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Introduction

The occurrence of hybridization between invasive, non-native and native species has increased as invasive species have become more prevalent (Ayres et al., 1999). Many issues related to biodiversity are posed by hybridization between native and non-native species, including the potential for the new hybrid to be more invasive than its parental species. Hybridization has the potential to degrade species diversity to the point of extinction either by "swamping out" native gene pools or by competitive displacement (Rhymer and Simberloff, 1996). Ayres et al. (1999) suggested that hybrids between the non-native (*Spartina alterniflora*) and the native (*S. foliosa*) cordgrass may be displacing the native species in western USA. This conclusion is tentative, however, due to the difficulty of distinguishing the hybrid from its parental species. In this case and others, morphological similarity may cause a problem in accurate estimation of hybrid abundance.

Morphological similarity and a lack of isozyme variation have not only led to difficulties in identification of Ohio's cattail taxa, but has resulted in a lack of knowledge concerning hybrid frequencies and inter-taxon gene flow. Three taxa of cattail (*Typha*) are generally recognized in Ohio. *Typha latifolia* (broad-leaved cattail), a North American native, is common in wetlands throughout much of the United States (Figure 1). *Typha angustifolia* (narrow-leaved cattail), thought to be introduced from Europe (Smith 2000), is located in the northeastern range of *T. latifolia* and is considered an invasive species due to its spreading range, and its ability to successfully establish monocultures that displace native plants (Figure 1). The hybrid of the two species, *Typha Xglauca*, occurs within the range of *T. angustifolia* and may invade areas not previously inhabited by the parental species, such as eutrophic and disturbed habitats with unstable water levels (Smith, 2000).

The status of *Typha Xglauca* has long been disputed and relatively little is known about the genetic variation, taxonomy and ecology of this plant. Previous research has concentrated on morphological and isozyme variation to identify the three taxa. Hotchkiss and Dozier (1949) recognized *Typha Xglauca* as a separate species from of *T. latifolia* and *T. angustifolia*, while Fassett and Calhoun (1952) presented morphological evidence of introgression, believing that *Typha Xglauca* represented a series of intermediate individuals in a hybrid swarm. Smith (1967) found that hybrids could be artificially produced between *T. latifolia* and *T. angustifolia*. He also found that F1 hybrids commonly occur in habitats where the parental species do not grow (Smith, 1967, 1987). Lee (1975) found evidence for hybrids using isozymes, but thought both *T. angustifolia* and *Typha Xglauca* were more similar to the southern cattail species *T. domingensis* than to *T. latifolia*. In a more recent study of isozyme variation among *Typha* species, Sharitz et al. (1980) found species-specific alleles at three allozyme loci. Their data revealed that hybridization was not presently

Figure 1. Distribution maps of *Typha latifolia* (left) and *T. angustifolia* (right) in North America (Smith 2000).

occurring in the populations sampled, and they concluded that *Typha Xglauca* was not a separate species, but an intermediate between *T. latifolia* and *T. angustifolia*. (Sharitz et al., 1980).

Kuehn et al. (1999) used molecular markers to examine hybridization between cattails. Species-specific random amplified polymorphic DNA (RAPD) markers were developed, and hybrids contained bands from both species. Kuehn et al. (1999) surveyed cattails throughout the northern Great Lakes Basin, southern Canada and Great Britain and reported that although hybrid frequency in nature may be extensive, mature hybrid stands are only of the F1 generation. In a later study, Kuehn and White (1999) used genetically identified specimens of each species to discriminate reliable taxonomic characters such as stigma width, spike length, spike gap and leaf width for morphological identification. In their study, use of morphological characters corresponded to the genetic identity 90% of the time. This method may be useful for researchers and land managers who do not have the resources for DNA analysis, but are interested in which cattail species are present at a given site. The goals of this study were to identify the cattail taxa present at an artificial wetland and to document the potential for interspecific hybridization by investigating flowering phenology.

Materials and Methods

Study Site

The Olentangy River Wetlands Research Park (ORWRP) at The Ohio State University includes man-made wetlands used for scientific study. Created in 1994, the wetlands' first cattails arrived by seed in August 1994. The cattail composition of the wetlands has not been studied, but reports based on morphology indicate that all three taxa are present (Mitsch et al., 1998). Both *T. latifolia* and *T. angustifolia* are common at ORWRP, although *T. angustifolia* is more abundant (S.M.S. pers. obs.).

Variation in Phenology

In May 2000, ten plots measuring 0.5 x 2 m were chosen along the boardwalks at the ORWRP Wetland 2 to assess phenological differences between the cattail species. Plots were chosen that contained both species based on leaf width, leaf color, presence of spike gap and clonal density (Table 1). Flowering times were recorded three times a week by observing all individuals that were flowering in each plot. Female flowers of cattail open first, with male flowers releasing pollen only after the female flowers begin to senesce. Once flowering, each plant was identified to species and recorded as to whether the female or male flowers were receptive.

Variation in Phenotypic Traits

Morphological measurements were taken throughout the growing season to assess intra-specific variation. These measurements included pollen type, spike gap distance, leaf width, spike width and spike length. Approximately 100 flowering shoots were randomly chosen throughout the wetland and flagged in June 2000. Width of the largest leaf and spike gap, width and length were measured. Pollen samples were collected from 40 of the marked plants and observed with Alexander's stain under a compound microscope for presence of monads, dyads, triads, tetrads or a combination of pollen types. Species designations were made first based on the DNA evidence and pollen type, then on the presence of a gap and leaf width. Differences in morphological measurements were compared between species using t-tests.

RAPD Analysis

Leaf tissue samples were collected from the 100 flagged plants and DNA was extracted from a subset of the individuals. Reaction mixtures and DNA amplification were based on the procedure outlined by Kuehn et al. (1999). Primer OPA-02 (Operon Technologies Inc.) was used for amplification based on the success Kuehn et al. (1999) had with that particular primer.

Table 1. Morphological and ecological characteristics of three cattail taxa (all have chromosome numbers of n = 15) (Smith, 1967, 2000; Grace and Harrison, 1986).

	Typha latifolia	*T. angustifolia*	*T. Xglauca*
Leaf width	10-23mm	4-12mm	5-17mm
Leaf color	dark green	yellow-green	
Gap in floral spike	no	yes	yes
Height of floral spike relative to leaves	leaves equal spike	leaves exceed spike	
Pollen shed as	tetrads	monads	mixture
Salinity tolerance	mid	highest	low
Water depth	shallow	shallow or deep	
pH	basic to acidic	mostly basic	either

RAPD products were separated by electrophoresis on 1.2% agrose for 1 hour at 90 V. Gels were stained with ethidium bromide and visualized under UV light. Molecular weights of amplification products were estimated using a 1-Kb ladder and visually compared to results reported by Kuehn et al. (1999). Each gel was repeated at least twice.

Results

Very little overlap in timing of flowering spikes was observed between the two species (Figure 2). Although less pronounced in *T. latifolia*, both species display female receptivity before the staminate flower begins to shed pollen. *Typha angustifolia* flowered earlier in the season and for a longer time than *T. latifolia*. Most of the pistillate flowers of *T. angustifolia* had senesced before the staminate flowers of *T. latifolia* released pollen.

Significant differences between the two species are reported for gap size, leaf width and spike width (Figure 3). Spike length did not differ between the two species. Pollen from *T. angustifolia* (N = 21) was consistently shed as monads, while tetrads were shed from *T. latifolia* plants (N = 18).

The RAPD primer selected produced two species-specific bands for *T. angustifolia* and three species-specific bands for *T. latifolia*. Hybrids between the two species would contain both sets of bands (Kuehn et al., 1999). No such hybrids were found in my analysis of 30 individuals for the ORWRP. Furthermore, morphological measurements, pollen type and species-specific RAPD markers correspond in all but two individuals (Table 2).

Discussion

An understanding of hybrid frequencies between *T. angustifoila* and *T. latifolia* is useful for predicting the evolutionary future of these species. It is well documented that *T. angustifolia* is spreading outward into the range of *T. latifolia*; thus potentially displacing the native cattail. There is also indication that the hybrid of the two species is more vigorous and is able to colonize habitats where the parental species are not found (Smith, 1967, 1987).

It is difficult to assess the true effects of *T. Xglauca* on the native populations due to the fact that current taxonomic keys are unable to provide morphological features that clearly separate the parental species from the hybrid. Qualitative traits, such as leaf coloration, perhaps useful if the two species are next to each other, are subject to interpretation. Quantitative characteristics, although more useful than qualitative traits, widely overlap among the two parental species and the hybrid (Kuehn et al., 1999). Molecular techniques, such as RAPDs, are diagnostic for the two species at the ORWRP, and Kuehn et al. (1999) provided evidence that the hybrid could also be clearly identified with RAPD markers. Since the usefulness of molecular techniques can hardly cover the needs of land managers and researchers in the field, comparing morphological characters with the DNA markers may be helpful in determining which characteristics are diagnostic.

This study suggests that characteristics such as pollen, spike gap, leaf width and spike width can be used to discriminate between *T. angustifolia* and *T. latifolia* at the Olentangy River Wetlands Research Park. Although not

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Figure 2. Flowering Phenology in *Typha latifolia* and *T. angustifolia* (Ang). F indicates when female flowers are receptive and M indicates mate receptivity.

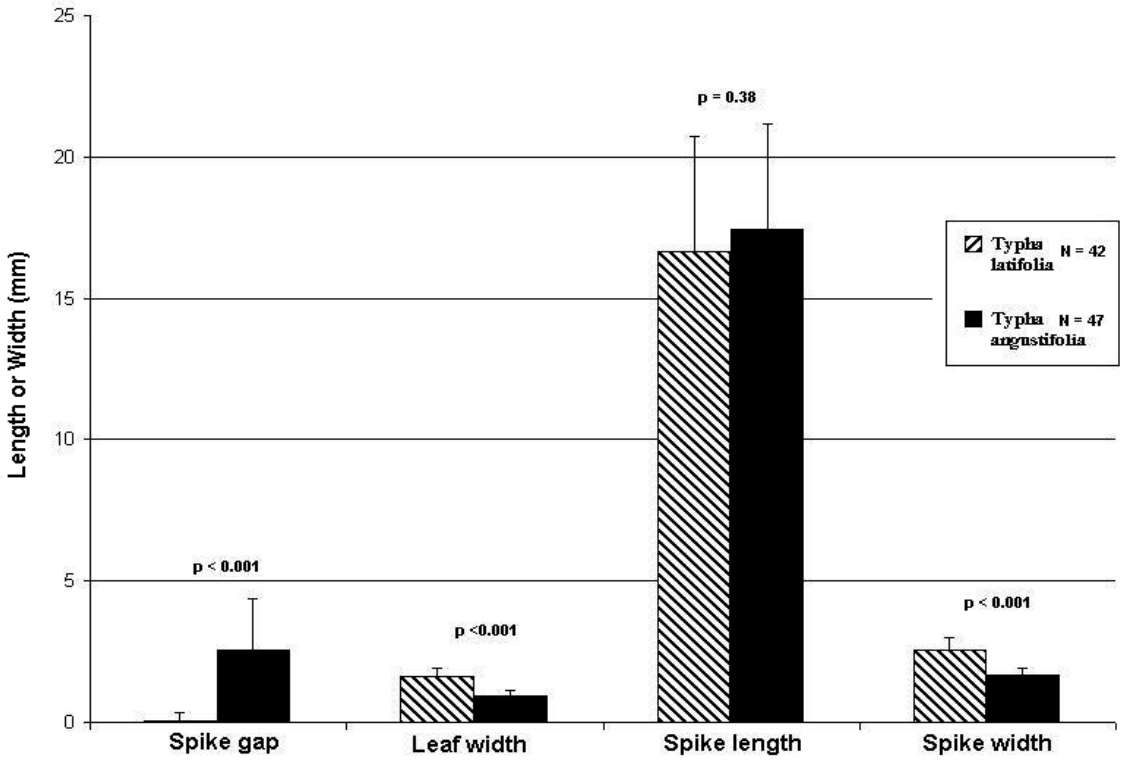

Figure 3. Morphological measurements comparing two cattail species. Error bars indicate standard deviations.

Table 2. Comparison of morphological and molecular traits. Number of plants in each category is listed. Note a indicates the same individual.

		RAPD Marker Type	
		T. angustifolia	*T. latifolia*
Pollen			
	tetrad	0	7
	monad	7	0
	other	0	0
Spike gap			
	present	20	1
	absent	1a	10
Leaf Width			
	< 13 mm	20	0
	≥ 13 mm	1a	11

100 percent accurate, morphological measurements are fairly reliable and are easily assessed during the flowering season. DNA evidence will prove to be most useful when shoots are not flowering or when hybrid status is suspected.

Although hybrids are not currently present at the ORWRP, these results indicate a potential for hybridization during the short overlap in flowering times between the two species. That hybridization was not revealed in this study may be due to several factors. Smith (pers. comm.) believes that hybrid seeds are largely inviable and successful hybrid plants are largely clonal. Kuehn et al. (1999) discusses similar possibilities, in that they only found F1 hybrids and never later generations in mature hybrid stands. It is possible that hybrids once existed at the ORWRP and did not survive, or perhaps the ecological conditions are not ideal for hybrid establishment. On the other hand, more time may be required for hybridization to occur (the wetland is less than 10 years old). Without a clear understanding of the hybrid cattail's ecology and invasiveness, it is difficult to explain its presence or absence.

What remains clear is that if *T. Xglauca* is more invasive than its non-native parent, stronger efforts will need to be taken to control the spread of *T. angustifolia* to eliminate the chance of hybrid formation with the native *T. latifolia*. Monitoring at the Olentangy River Wetland Research Park should continue in an effort to identify potential hybrids and study their ecology for potential invasiveness.

References

Ayres, D.R., D. Garcia-Rossi, H.G. Davis and D.R. Strong. 1999. Extent and degree of hybridization between exotic (*Spartina alterniflora*) and native (*S. foliosa*) cordgrass (*Poaceae*) in California, USA determined by random amplified polymorphic DNA (RAPDs). Molecular Ecology 8: 1179-1186.

Fassett, N.C. and B. Calhoun. 1952. Introgression between *Typha latifolia* and *T. angustifolia*. Evolution 6: 367-379.

Grace, J.B. and J.S. Harrison. 1986. The biology of Canadian weeds. 73. *Typha latifolia L., Typha angustifolia* L. and *Typha xglauca* Godr. Canadian Journal of Plant Science 66: 361-379.

Hotchkiss, N. and H.L. Dozier. 1949. Taxonomy and distribution of North American cattails. American Midland Naturalist 41: 237-254.

Kuehn, M.M., J.E. Minor and B.N. White. 1999. An examination of hybridization between the cattail species *Typha latifolia* and *Typha angustifolia* using random amplified polymorphic DNA and chloroplast DNA markers. Molecular Ecology 8: 1981-1990.

Kuehn, M.M. and B.N. White. 1999. Morphological analysis of genetically identified cattails *Typha latifolia*, *Typha angustifolia* and *Typha* x *glauca*. Canadian Journal of Botany 77: 906-912.

Lee, D.W. 1975. Population variation and introgression in North American *Typha*. Taxon 24(5/6): 633-641.

Mitsch, W.J., X. Wu, R.W. Narin, P.E. Weihe, N.Wang, R. Deal and C.E. Bouher. 1998. Creating and restoring wetlands. BioScience 48: 1019-1030.

Rhymer, J.M. and D.S. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27: 83-109.

Sharitz, R.R., S.A. Wineriter, M.H. Smith and E.H. Liu. 1980. Comparisons of isozymes among *Typha* species in the eastern United States. American Journal of Botany 67: 1297-1303.

Smith, S.G. 1967. Experimental and natural hybrids in North American *Typha* (Typhaceae). American Midland Naturalist 78: 257-287.

Smith, S.G. 1987. *Typha*: its taxonomy and the ecological significance ofhybrids. Archiv fur Hydrobiologie, Beih. Ergebn. Limnol. 27: 129-138.

Smith, S.G. 2000. Typhaceae. Flora of North America Volume 22. Oxford, New York.